Notes on the forest soil respiration measurement by a Li-6400 system

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Abstract: The correct method used in forest soil respiration measurement by Li-6400 is a premise of data quality control. According to the study in a larch plantation, collars should be inserted at 12 hours in advance to efficiently reduce the influence of CO_2 spring-out. Moreover, collar insertion depth substantially affected soil respiration measurement, i.e. when collar was shallowly inserted into soil, transversal gas diffusion and the CO_2 re-spring-out caused by unstable collars in the measurement could lead to overestimating soil respiration rate; however, when collar was deeply inserted into soil, root respiration decline caused by root-cut and the most active respiratory of the surface soil separated by the inserted collars could lead to underestimating soil respiration rate. Furthermore, an error less than 5% could be guaranteed in typical sunny day if the target [CO_2] was set to the mean value of ambient [CO_2] in most time of the day, but it should be carefully set in early morning and late afternoon according to changing ambient [CO_2]. This protocol of measurement is useful in real measurement.

Keywords: Li-6400; Soil respiration; Collar insertion depth; CO_2 spring-out effect; Gas transversal diffusion; Factory parameter selection

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Introduction

Different from other soils with naked surfaces, forests soil is generally covered by litter layer with varied depths. The CO_2 in soil springs out in a large magnitude just after disturbance of soil surface, which is usually called as CO_2 spring-out effect (LiCor 1997). In order to diminish this effect, thin-walled polyvinyl chloride (PVC) collars are recommended to be pre-inserted (Wang *et al.* 2005). Moreover, collar insertion may decline soil respiration by root-cutting (Wang *et al.* 2005) and also may hinder the diffusion of CO_2 from soil into or out of collar. But, to date, the magnitude of the hindrance effect is still not clear and the strength and duration of collar CO_2 spring-out from different soil depths and litter layer are also less known for a specific forest of larch. Therefore, study on the traits of CO_2 spring-out from forest soil and hindrance of diffusion by inserting collar is scientifically necessary.

Microenvironment in trunk space from forest floor to canopy is turbulent and complex (Green *et al.* 1995), and [CO_2] near soil surface in forest is fluctuated largely throughout the day and the season (Bazzaz 1996; Koike *et al.* 2001). This kind of changing environment at forest floor may influence the protocol of soil respiration measurement. In fact, the CO_2 flux chamber of Li-6400-09 is in a dynamic equilibrium since pressure differences greatly impact the estimate accuracy of soil respiration (Nakayama 1990; LiCor 1997) and the parameter of the system is also selectable. As soil respiration is measured, target [CO_2] is set to ambient [CO_2], and the target [CO_2]±delta [CO_2] is the range for soil respiration measurement. When chamber [CO_2] is higher than the target [CO_2]+delta [CO_2], chamber CO_2 will be diverged to soda lime until [CO_2] is lower than target [CO_2]-delta [CO_2]. Then respiratory CO_2 begins to accumulate in chamber and the linear relation between chamber [CO_2] with time will be monitored and the slope value is estimated as soil respiration rate (LiCor 1997). Thus, the selection of these parameters during measurement in fluctuating [CO_2] environment at the forest floor is important to ensuring data reliability. The question is that how often we should change these parameters in the diurnal measurement, which is less known for a specific forest (LiCor 1997). This question could be answered by checking on the precision of machine and range of CO_2 fluctuation in environment. Thus, one experiment on monitoring the CO_2 fluctuation in forest floors was carried out. The other experiment of changing target [CO_2] with a fixed delta [CO_2], and changing delta [CO_2] with a target [CO_2] were used to testify the precision of Li-6400. Combining these two experiments, the possible protocol of parameter (delta CO_2 and target CO_2) selection on soil respiration measurement in forest floors was proposed.

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Study site and methods

Study site

A larch forest (*Larix gmelinii*) (planted in 1969) at Laoshan station of Mao'ershan Experimental Forest Farm was selected as the study site (N45°20′, E127°34′). The mean height and diameter at breast height (DBH) are 18 m and 17.2 cm, respectively. The mean annual precipitation is 723 mm. The annual mean air temperature is 2.8 °C. Stand density is 1100 individual·hm^{-2}. Forest soil is typical dark-brown forest soil (Inceptisols or Andisols). The pH is about 6.0 and soil organic matter is 8%–9%.

Experiment of CO_2 spring-out effect from litter and soil

For assessing the CO_2 spring-out effect after collar insertion into different soil depths, four collar-insertion depths (0.3, 2.3, 4 and 5 cm into litter) were settled and soil respiration at 6 time

intervals (0, 3, 6, 9, 24, 30 hours) at each insertion depth were measured. Considering the 2-cm thickness of litter in the study site, the first treatment is actually above the litter, so we called it as "above litter" treatment in further discussion. The other three treatments were actually at soil depths of 0.3 cm, 2 cm and 3 cm. A Latin square experimental design was used for avoiding an experimental error between different rows and columns (Fig. 1A). For each insertion depth, four replicates were measured.

The duration of CO_2 spring-out was modeled using the following equation:

$$Y = Y_0 + A_1 e^{(-x/t_1)} \quad (1)$$

where, Y and X are soil respiration rate and time, respectively, Y_0, A_1 and t_1 are constant. The sum of Y_0 and A_1 implies the maximum soil respiration rate.

The decreasing rate of the CO_2 spring-out effect (D) is the absolute value of the derivative form of Equation (1), described by following Equation (2)

$$D = \frac{A_1}{t_1} e^{(-x/t_1)} \quad (2)$$

when t_1 is 0 (just after disturbance from collar insertion), D equals to $\frac{A_1}{t_1}$. It indicates the strength of CO_2 spring-out effect and we called it as D_{initial} in further discussion (Fig. 3). When D is lower than 1%, we assume that no spring-out effect occurred in the soil respiration measurement. This time point (T) could be calculated by the Equation (3), derived from Equation (2):

$$T = -t_1 \ln\left(\frac{t_1}{A_1}\right) \quad (3)$$

Where, T is the duration of CO_2 spring-out after disturbance of collar insertion since the initial time of the disturbance is 0. We use T_{duration} as an abbreviation in further discussion (Fig. 3).

Experiment of CO_2 diffusion hindrance by collar insertion

In order to assess the influence of CO_2 diffusion hindrance by collar insertion on the soil respiration measurement, 2 types of collars (5 cm and 10 cm in length) were chosen to be inserted 8-cm depth into the soil. In the case of the 5-cm collar, two collars were overlapped together to insert into the soil to ensure the 8-cm insertion depth (Fig. 1C). Gas diffusion might have taken place at the edge between the two collars when the soil respiration measurement was carried out. Two types of collar were set every other to avoid the experimental error between different rows and columns (Fig. 1C). The collars were preset at least 12 h before soil respiration measurement.

Furthermore, with the insertion depth increase, fine root respiration can substantially decline because their activity can be sharply inhibited by root-cut from collar (Wang *et al.* 2005). For excluding this root-cut effect, soil respiration rate was measured both in control (with root) and trenched box (without root) and the CO_2 diffusion hindrance was demonstrated by variable insertion depths (Fig. 1 A&B). Experiment in trenched box was done in three months after trenched box was made. This duration is longer enough to neglect the influence of root debris decay on bulk soil respiration (Lee *et al.* 2003). Root vertical distribution showed that most of the roots were within the soil depth of 0–40 cm in our study site. Therefore, our trenched boxes with 50-cm soil depth could be enough to inhibit root regrowth. Trenched boxes were prepared by making vertical depth into the ground surface about 50 cm using a steel knife and shovel.

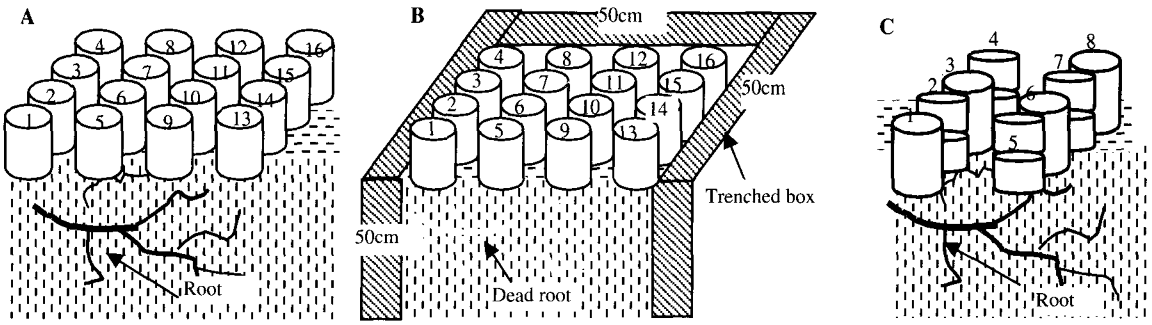

Fig. 1 Experiment designs of the collar insertion depth in control (A) and trenched box (B), and gas diffusion hindrance by inserting collars (C)
The Latin square experimental design was used in panel A and B, the collars at No. 1, 8, 11 and 14 were inserted 0.3-cm depth into soil; the collars at No. 2, 5, 12 and 15 were inserted 2-cm depth into soil; the collar at No. 3, 6, 9 and 16 were inserted 5-cm depth into soil; the collar at No. 4, 7, 10, 13 were inserted 8-cm depth into soil. In panel C, the collars at No. 1, 3, 6 and 8 were inserted 8-cm depth into soil with one collar, therefore, no gas leakage from collar wall into collar happened. The collars at No. 2, 4, 5 and 7 were inserted 8-cm depth into soil with 2 overlapped collars, therefore, gas leakage occurred at the connection edge of two collars.

Protocol determination of delta and target CO_2 settings in diurnal fluctuating $[CO_2]$ at forest floor

Six different delta $[CO_2]$ values (1, 5, 10(default), 15, 20, 25 μmol · mol^{-1}) were set to evaluate the influences of delta $[CO_2]$ differences on the final results. Seven target $[CO_2]$ values (330, 360, 370, 380, 390, 400 and 430 μmol·mol^{-1}) were set to evaluate the influences of the target $[CO_2]$ differences on the soil respiration measurements. We concurrently measured the $[CO_2]$ concentration in the forest floor of the study site (380 μmol·mol^{-1}). This value was set as the ambient $[CO_2]$ in the measurements.

For evaluating the daily variation in $[CO_2]$ and their possible request for parameter selection of Li-6400 in respiration measurement, we also measured the diurnal course of $[CO_2]$ near forest floor by Li-6400 with broadleaved chamber from early morning (6:00) to late afternoon (18:00) on 4 typical sunny days. Data were automatically logged by the autoprograme. Different

from leaf photosynthesis measurement, no leaves were clamped in this measurement and no air buffer tank was used.

Data analysis

Relative value of respiration rate was used in this study to avoid the influence from soil heterogeneity. In the experiment of CO_2 spring-out, we used the mean respiration value of each collar measured just after collar insertion (time point 0) as 100%. Accordingly, relative values at other timepoints were computed. For comparing the root-cut effect and diffusion hindrance by collar insertion in control and trenched box plots, we used the mean respiration value of treatment at 0.3-cm soil depth as 100% and accordingly, relative values at other insertion depths was calculated. Best-fit regression was carried out by Microcal Origin 7.0. Statistical analyses were carried out by SPSS 10.0.

Results and discussion

The duration and strength of CO_2 spring-out from forest litter and bulk soil

Soil respiration exponentially decreased with elapsed time in all our treatments with different collar insertion depths (Fig. 2). The decreasing rates of CO_2 spring-out were different with different collar insertion depths (Fig. 3). $D_{initial}$ from the "above litter" treatment was higher than that for other treatments. However, $T_{duration}$ for this treatment was shorter than that for all other treatments (Fig. 3). Therefore, the CO_2 spring-out from "above litter" treatment was stronger and quicker. For example, $D_{initial}$ from the "above litter" and at soil depth of 0.3 cm, 2 cm and 3 cm were 11.5, 6.4, 5.3 and 5.9 percent·h^{-1}, respectively. The duration of the spring-out effect of the above mentioned treatments were 10.2, 14.5, 12.8, 12.1 hours, respectively (Fig. 3b).

The different traits of CO_2 spring-out from forest litter and different soil depths should be considered in soil respiration measurement in forest soil. When collar was above litter, the transversal CO_2 transport and re-spring-out from the unstable collar was more serious than that in other treatments, which resulted in about 2 times stronger CO_2 spring-out effect ($D_{initial}$). However, the duration of CO_2 spring-out was about 2 hours shorter than that in the treatments with collar inserted into soil. Generally, it is necessary to insure the data quality at least 12 hours pre-insertion when the collar is inserted into soil surface.

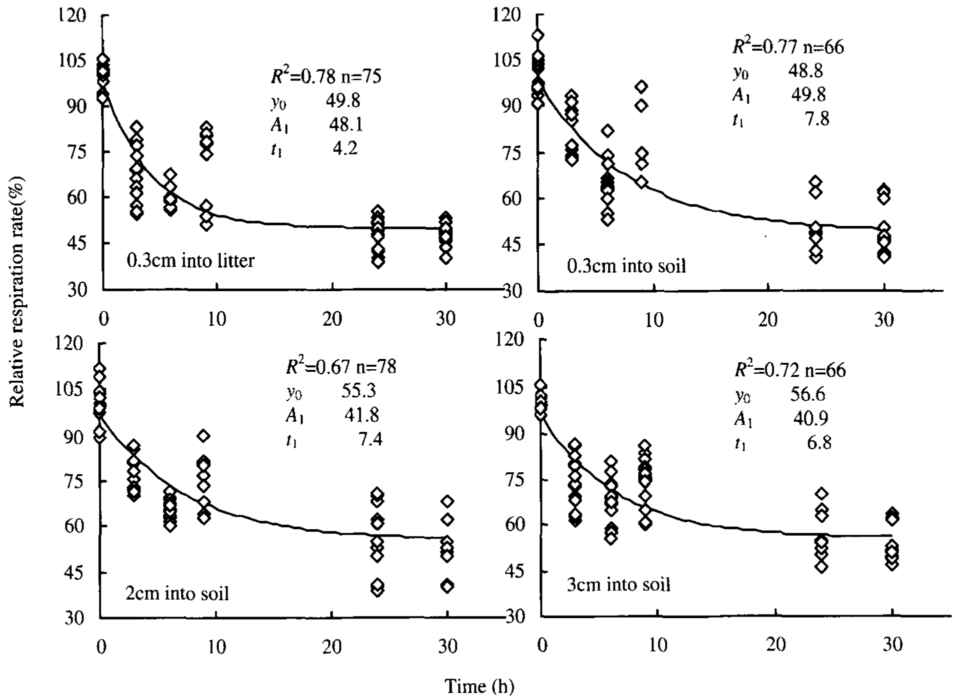

Fig. 2 The duration of the CO_2 spring-out effect and possible relationship with collar insertion depth

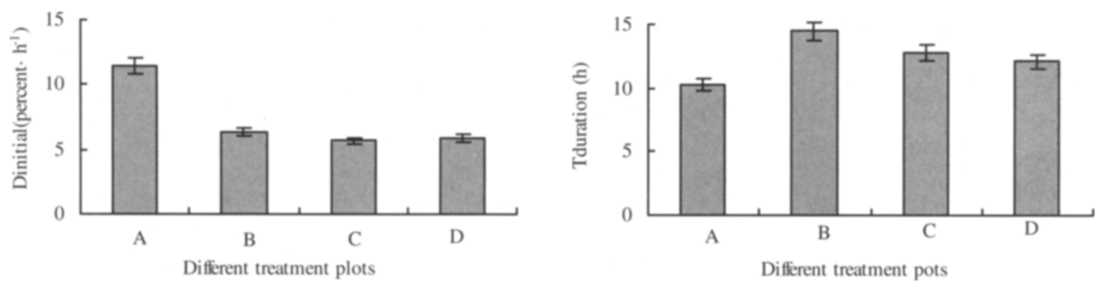

Fig. 3 The strength of CO_2 spring-out, $D_{initial}$ (a) and duration of CO_2 spring-out, $T_{duration}$ (b) in different treatment plots (A, B, C and D are above litter (about 0.3 cm into litter), 0.3 cm into soil, 2 cm into soil and 3 cm into soil, respectively. The vertical bars show an error of 5%)

Gas transversal diffusion and root-cut effect by collar insertion

With insertion depth increase, soil respiration both in control and trenched plots declined, however, the steepness of the decrease was substantially different (Fig. 4 A), i.e. steepness in the control plot (with root) was higher than that in the trenched box plot (without root). The root, especially fine root cut by collar was significant correlated with the respiration decline, indicating that decline in fine root respiration may be responsible for this fast decline of total respiration rate in "with root" plot (Wang *et al.* 2005). However, the reason for the decrease in trenched plot (without root) was not clearly explained. To fully understand this slow decline of soil respiration, another experiment with and without diffusion at collar walls was carried out in our study site (Fig. 1C). It was found that marked increase in soil respiration was observed when soil CO_2 leaked from the collar wall into the collar with 8-cm insertion depth (p<0.05) (Fig. 4B). So, the transversal transport of CO_2 by gas diffusion significantly influenced soil respiration measurement. It seemed that no gas leakage out of the collar wall was found in our study. The reason may be the fact of the decrease in root respiration within the collar wall.

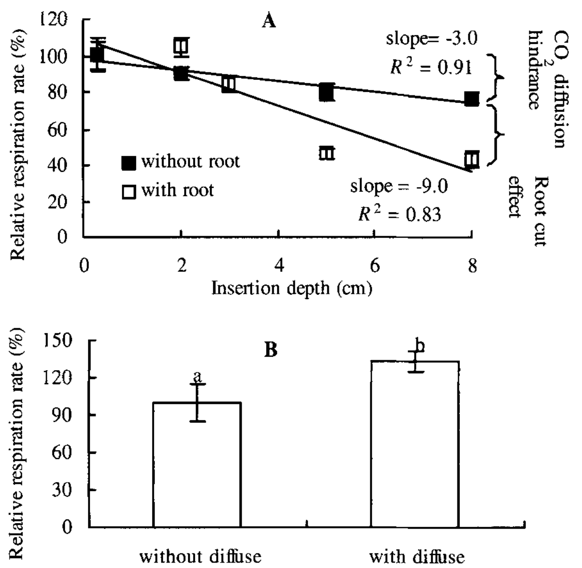

Fig. 4 Soil respiration rate decreases with collar insertion depth in with root and without root treatments (A) and difference in respiration rate when two types of collar were used in the measurement (B) (The vertical bar on each column indicates the standard error of mean value. Different letters on the columns indicate the difference between two values is significant (p<0.05))

Collar insertion can substantially reduce the CO_2 spring-out, but the insertion can decrease root respiration with cutting root (Fig. 4 A) and also can change the CO_2 transversal diffusion in soil and litte Very deep collar insertion into soil can decline root respiration by root-cut, and very shallow collar insertion will accelerate the transversal gas diffusion and CO_2 re-spring out from the unstable collars. Therefore, the collar insertion depth should be carefully considered.

However, in the reported materials, little attention is paid to this point. The insertion depth is less than 0.5 cm in some study (Widen *et al.* 2001), and about 4–5 cm in some other studies (Mariko *et al.* 2000; Lee *et al.* 2002), even 10.5-cm insertion depth is also reported (Irvine *et al.* 2002). Moreover, the influece from the penetration of litter layer is not fully considered, even in a needle leaved forest (Widen *et al.* 2001) (whose litter is difficult to penetrate). Generally, variances of soil respiration were attributed to biotic and abiotic factor (Buchmann 2000), but few of studies consider the collar insertion effect. Our finding indicates that collar insertion depth differences maybe another possible reason for large variances in soil respiration.

Fig. 5 is a sketch map on the influences of collar insertion on soil respiration measurement. With the insertion depth increase, more roots could be cut, which may reduce root respiration in a long run. Moreover, this insertion may exclude the surface soil respiratory CO_2 into the measurement. Since the surface soil is the most active soil layer for respiration, this respiratory exclusion of surface soil by the inserting collars also decreases the apparent rate in the respiration measurement, which has been found in the trenched soil plot (without root) as shown in Fig. 4 A.

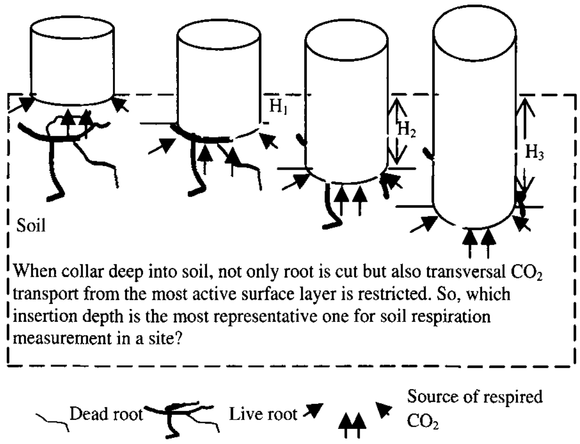

Fig. 5 Sketch map of the influences of depth of collar insertion on soil respiration by root-cut effect and CO_2 transversal transport from surface soil layer (H_1, H_2 and H_3 are the depth of collar insertion. With this insertion decrease, more CO_2 respired from the surface soil can be included into soil respiration measurement)

Protocol for respiration measurement in fluctuating [CO_2] at forest floor

Target [CO_2] and delta [CO_2]) should be decided when soil respiration measurement was done by a Li-6400 (Table 1). For understanding this influence of fluctuating ambient [CO_2], in the following discussion, different target and delta [CO_2] were selected in the measurement. We found that lower target values generally caused a higher estimation of soil respiration rate and higher target values caused a lower soil respiration rate. However, no statistical difference was observed if the deviation of target [CO_2] from ambient [CO_2] was less than 10 μmol·mol^{-1}, and no major differences (<5%) were observed if the target [CO_2] did not deviate far from ambient [CO_2] (<20 μmol·mol^{-1}) (Table 1). But, when target value was deviated far from the ambient [CO_2], large differences were observed (Table 1). Furthermore, comparing the factory default delta [CO_2] (10 μmol·mol^{-1}) of the Li-6400, the higher delta [CO_2] generally caused a lower estimate of soil respiration, but no statistical differences were found among the measurement (Table 1). Compared to the influence from target [CO_2], delta [CO_2] values had a slight influence on the soil respiration measurement. All the errors resulting from the delta [CO_2] were quite small (Table 1). But big delta [CO_2] generally causes a long interval in measurement,

especially in the case of quite low respiration rate. Thus, a reasonable delta value should be selected in measurement.

Table 1. Influences of the selection of factory parameters (variation of target CO_2 with a fixed delta CO_2 of 10 mol·mol^{-1}and variation of delta CO_2 with a fixed target CO_2 of 380 mol·mol^{-1}) on the respiration measurement

Target CO_2 (mol·mol^{-1})	R (mol·m^{-2}·s^{-1})	(R-$R_{ambient}$) /$R_{ambient}$(%)	Delta CO_2 (mol·mol^{-1})	R (mol·m^{-2}·s^{-1})	(R-$R_{default}$) /$R_{default}$ (%)
330	4.3a	17.5	1	2.03a	2.8
360	3.88b	6.3	5	1.94a	-1.8
370	3.80bc	4.2	10 (default)	1.97a	0
380 (ambient)	3.65cd	0	15	1.94a	-1.3
390	3.61cd	-0.8	20	1.90a	-3.7
400	3.55d	-2.7	25	1.91a	-3.1
430	3.13e	-14			

Notes: R----Respiration; (R-$R_{ambient}$) /$R_{ambient}$---- under- or over-estimation percentage.

In typical sunny days, [CO_2] at forest floor was relative stable in day time except early morning and late afternoon (Fig. 6). The [CO_2] from 8:00 to 17:00 were within the range from 350 μmol mol^{-1}to 390 μmol·mol^{-1} (370 μmol·mol^{-1}±20 μmol·mol^{-1}) and the [CO_2] from 9:00 to 15:00 were from 356 μmol·mol^{-1}to 374 μmol·mol^{-1} (365 μmol·mol^{-1}±10μmol·mol^{-1}) without marked difference (p<0.05), (Fig. 6). So, in this period of time in typical sunny days, an error less than 5% can be guaranteed by one suitable target [CO_2] alone in soil respiration measurement. However, in early morning and late afternoon, the [CO_2] were dramatically fluctuated in Fig. 6. Thereby, the measurement value was seriously influenced if same target [CO_2] were used.

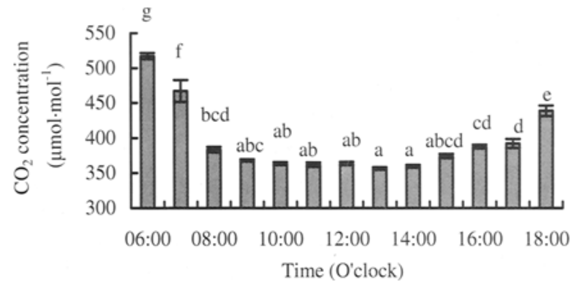

Fig. 6 Diurnal course of CO_2 concentration near soil surface (ca. 50 cm in height) in the forest (The vertical bars on the columns indicate the standard error of the mean value. Different letters indicate the difference between these columns are significant (p<0.05))

In practice of soil respiration measurement, due to the disturbance from the operator of Li-6400, the ambient [CO_2] is not so easy to be precisely estimated when soil respiration is measured. Thus, although LiCor Company requests that the target [CO_2] should be set to ambient [CO_2] at each measurement (LiCor 1997). The exchange of target [CO_2] in every measurement is not feasible and practicable due to the difficulty in real-time measurement of [CO_2]. Our finding indicates that, when we understand the pattern of [CO_2] at forest floor, we can use one unique target [CO_2] in most time of typical sunny days.

Conclusion and suggestion

The duration and strength of the CO_2 spring-out from the forest litter and bulk soil had different patterns. Therefore, the pre-setting of the collar should be done at least 12 hours before the measurement is taken in order to ensure the CO_2 spring-out effect (<1%). Collar insertion depth had a significant influence on soil respiration measurement. Therefore, collar should be inserted into soil with appropriate depth to avert root-cut and gas transversal diffusion hindrance. Very deep insertion into soil can decline root respiration by root-cut, and very shallow insertion will mistake the rate of respiration by CO_2 re-spring-out and gas leakage from the unstable collar.

In typical sunny days, dramatic fluctuation in [CO_2] in the forest floor was observed in early morning and late afternoon, but relative stable values (8:00 to 17:00: 370±20 μmol·mol^{-1}; 9:00 to 15:00:365±10 μmol·mol^{-1}) were observed in the rest time. Thereby, one target [CO_2] was used in most time of the day, but the target [CO_2] should be carefully chosen in early morning and late afternoon. This type of protocol of measurement is feasible and practicable in real measurement.

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